AD NUMBER

AD408629

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; May 1963. Other requests shall be referred to Army Biological Lab., Fort Detrick, MD 21701.

AUTHORITY

SMUFD d/a ltr, 8 Feb 1972

AD 408 629

TESTS WITH PASTEURELLA PSEUDOTUBERCULOSIS AND PASTEURELLA PESTIS BACTERIOPHAGE

TRANSLATION NO.

802

MAY 1963

U.S. ARMY BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

CCBL: FD3-3957(T-77-1)
JPRS: R-3121-D

10 May 1963

TESTS WITH PASTEURELLA PSEUDOTUBERCULOSIS AND PASTEURELLA PESTIS BACTERIOPHAGE

Translated for:

U. S. CHEMICAL CORPS BIOLOGICAL LABORATORIES
Ft. Detrick, Md.

By:

U. S. DEPARTMENT OF COMMERCE
OFFICE OF TECHNICAL SERVICES
JOINT PUBLICATIONS RESEARCH SERVICE
Building T-30
Ohio Drive & Independence Ave., S.W.
Washington 25, D. C.

TESTS WITH PASTEURELLA PSEUDOTUBERCULOSIS AND PASTEURELLA PESTIS BACTERIOPHAGE

/Following is the translation of an article by Werner Knapp of the Hygiene Institute at the University of Tubingen (Director: Prof. Dr. R. E. Bader) in Zeitschrift fur Hygiene the German-language publication (Journal of Hygiene), Vol 148, 1962, pages 375-383./

Through tests done by Flu (1927), Girard (1942, 1943), and Flu & Flu (1946), bacteriophage strains were discovered which have a lytic effect on individual strains of Escherichia coli, Shigella dysenteriae, and Pasteurella pseudotuberculosis.

Among other authors who have dealt with the lytic action of bacteriophage strains on Past. pseudotuberculosis (Past. pstbc.) and other types of bacteria, Sugino (1932), Advier (1933), and Bezsonova & Co. (1938), were unable to determine this property in their bacteriophage strains, while Lazarus and Gunnison (1947) and Gunnison and Lazarus (1948), in transfering one of the bacteriophage strains which had been tested by Advier (1933) on Past. pstbc. found that 31 out of 40 underwent lysis prior to the adaptation, and all of the strains tested lysed after the adaptation. Out of 42 strains of Salmonella which represented 25 different species, 3 strains (one each of Salm. schottmueller, Salm. hirschfeldii, and Salm. rubislav) and out of 37 strains of Shigulla, 6 strains were sensitive to concentrated suspension of bacteriophage. The attempts of Gunnison and Co. (1951) to differentiate between Past. pestis and Past. pstbc. with the aid of the same strain of bacteriophage and applying a critical testing dose at incubator temperatures of 22°C, resulted in a lysis of pestis tuberculosis strains; however, it did not affect any strains of pseudo-tuberculosis.

Apart from the above transfers of bacteriophage on Past. pstbc., there are no extensive tests with Past. pstbc. bacteriophage. Girard's report (1942) that some Past. pstbc. strains show the same sensitivity to Past. pestis bacteriophages as to Past. pstbc. bacteriophages fails to give more detailed data. Recently, during a small epidemic of pseudotuberculosis among marmots, Plankina and Ogneva (1961) succeeded in isolating the irritant and in finding bacteriophage strains in four Past. pstbc. strains. These phages, however, showed a reaction only with Past. pstbc. strains and a pestilence vaccine strain, but none with strains of Salmonella, Listeria, and Coli. The Past. pstbc. strains, cultured during the epidemic, in addition were lysed by a Past. pstbc. bacteriophage strain which was described by Kotliarova (1956).

Following our unsuccessful attempts to isolate phages in strains of human and animal origin which were cultured at the Institute of Hygiene, or received from other institutes for successive testing, we tried

to obtain a Past. pstbc. bacteriophage strain from elsewhere. Dr. Girard (Pasteur Institute, Paris) was kind enough to supply a bacteriophage strain (Y phage) and a Past. pstbc. bacteriophage strain which, according to reports (Girard 1960), had never been in contact with Past. pestis.

([Note] Unfortunately, until now we have failed to obtain the bacteriophage strains described by Kotliarova (1956) or Plankina and Co. (1961) for the purpose of extending our tests.)

This article reports on our tests in answering the following questions:

I. Does the PST-bacteriophage strain have an uniform effect on Past. pstbc. strains of serological types I - V, or can we prove clear differences in the sensitivity of individual strains of the same or different serological types to this phage?

II. Are there differences in the lytic action of the PST-phage strain on strains of Past. pstbc. and Past. pestis, following separated concentration of the original phage suspension over Past. pstbc. or Past. pestis?

III. What is the lytic action of the bacteriophage Y on strains of Past. pstbc., following separated concentration of the original phage suspension over Past. pestis and Past. pstbc.?

IV. Can the PST-phage strain, as the bacteriophage Y, be transfered to strains of Salmonella; is this transfer particularly possible to Salmonella strains which have antigenic relations to Past. pstc.?

V. Is it possible to adapt Salmonella phages to Past. pstbc., and how does a Salmonella phage strain which has been transfered on Past. pstbc. compare with strains of Salmonella, Past. pstbc., and Past. pestis?

Materials Used in Testing.

A. Pasteurella Phages. PST-phage: Past. pstbc. bacteriophage strain; supplied by Dr. Girard, Pasteur Institute, Paris.

Y-phage: Bacteriophage strain Y; supplied by Dr. Girard, Paris.

B. Salmonella Phages. Salmonella Typhimurium phage 1: supplied by Prf. Dr. Brandis, Institute of Hygiene, Gottingen.

Salmonella O-1 phage: supplied by Prof. Brandis, Institute of Hygiene, Gottingen.

C. Bacteria Strains. Past. pseudo-tuberculosis: testing strains of serological types I to V (No 2^{I}, 16^{II}, 43^{III}, 2200^{III}, 32^{IV}, Ikegaki^{IV}, 25^{V}, and $74R^{I}$), as well as other Past. pstbc. strains mentioned in this text are from the collection of the Institute of Hygiene, Tubingen.

Past. pestis: strains of avirulent pestis described as A1122, EV 76, and TWJ; supplied by Prof. Dr. K. F. Meyer, San Francisco.

Salmonella strains: Strains from the collection of the Institutes of Hygiene in Tubingen and Gottingen (Prof. Dr. Brandis), see test for particulars.

D. Culture Medium. Bacto-proteose-agar or solution, Difko (Difko-Manual 1953) for the purpose of conducting bacteriophage experiments on strains of Past. pstbc. and Salmonella.

Bacto-tryptose-agar, Difko (Difko-Manual); Tryptose-bouillon (Pepton 20 g (Merck), cooking salt 5 g, dextrose 1 g, broth 1000 ml) for the purpose of conducting bacteriophage experiments on Past. pestis.

([Note]: Our sincere thanks to Prof. Dr. K. F. Meyer, Prof. Dr. Brandis, and Dr. Girard, for their kind cooperation in supplying the strains, and to Prof. Dr. Brandis for his valuable advice during the experiments dealing with question IV.)

Method.

The transfer of phages to the various strains of bacteria and the successive concentration, unless otherwise mentioned, was done by means of the usual liquid or solid culture media (bibl. by Brandis 1957, Adams 1959, a. o.). As a rule, the Oese method was used to test the phage suspension. The Overlay method was used only for individual control tests.

Tests to Question I.

Following isolated concentration of the PST-phage over 7 different test strains of Past. pstbc. type I - V (strain No 2^{I}, 16^{II}, 43^{III}, 2200^{III}, 32^{IV}, Ikegaki ^{IV}, and 25^{V}), the 7 bacteriophage concentrations were tested against 42 Past. pstbc. strains, that is, 14 strains of type I, 10 strains of type II, 5 strains of type II, 4 strains of type IV, and 9 strains of type V. All bacteriophage concentrations which were transfered with an oese to cultures pre-hatched for $\frac{1}{2}$ to 1 hour, lead at a minimum dilution of 10^{-4} to a confluent lysis of the strain used for the concentration, and at a dilution up to 10^{-6} or 10^{-9}, to numerous or sporadic holes (philques) in the liquid area. All bacteriophage suspensions were concentrated, and tested at a dilution from 10^{-1} to 10^{-3}. A critical testing dose, that is, a concentration which produces a confluent lysis on the homologous strain during the application was not used in these tests.

Bacteriophage suspensions, concentrated on the Past. pstbc. strain 2^{I}, 16^{II}, 32^{IV}, Ikegaki ^{IV}, and 25^{V} and diluted up to 10^{-3}, lead to a confluent lysis in most Past. pstbc. strains of type I, II, III, and V. The 4 Past. pstbc. strains of type IV showed only numerous or sporadic plaques, without leading to a confluent lysis. The sensitivity of the Past. pstbc. strains type I, II, IV, and V, was noticeably smaller than that of the bacteriophage suspensions concentrated on Past. pstbc. strain No 43^{III} and 2200^{III}, which in most strains lead to a confluent lysis or plaques when concentrated or at a dilution of 10^{-1}, while the strains of type III used for concentration attained a confluent lysis even at a bacteriophage dilution of 10^{-4}.

Within the Pasteurella genre, only 3 avirulent pestis strains reacted to the PST-phages concentrated on the Past. pstbc. strain No 2^{I}. There was no reaction in 18 Past. multocida and 4 Past. tularensis strains. No effect of the PST-phages could be proved in 20 Coli, 12 Shigella, 5 Pseudomonas or 5 Klebsiella, and 6 Hemophilus strains.

In answering the first question, we can state that the PST-phage possesses a great specific effect on Past. pstbc. strains, and represents an additional aid in type diagnosis. In countries with the occurrence of pestilence, however, a differential diagnosis of Past. pestis (for example, according to the method of Gunnison and Co. 1951) should be taken into consideration. There are differences in the sensitivity of the individual strains of Past. pstbc. to the various concentrations of PST bacteriophage; however, all strains of serological types I-V were grasped by the PST-phage.

The questions still remain open as to whether our observations of the PST-phage apply to other Past. pstbc. bacteriophage strains as well, and whether the transfer of PST-phage to sutiable Past. pstbc. strains and the discoverey of further phage strains make a lytic typification of Past. pstbc. strains possible.

Tests to Questions II and III.

Concentrations of the PST-phage over Past. pstbc. strain 2^I (titer: confluent lysis up to 10^{-5}, numerous or sporadic plaques up to 10^{-7} or 10^{-9} of bacteriophage dilution) andover Past. pestis strain A 1122 (titer: confluent lysis up to 10^{-9} numerous or sporadic plaques up to 10^{-11} of bacteriophage dilution) or concentrations of the Y-phage over Past. pestis strain A 1122 (titer: confluent lysis up to 10^{-5}, numerous or sporadic plaques up to 10^{-8} or 10^{-10}), Past. pstbc. strain 2^I (titer: confluent lysis up to 10^{-7}, numerous or sporadic plaques up to 10^{-11}) were tested (as in I) against 42 Past. pstbc. strains and 3 Past. pestis trains.

We received the results as briefly summerized below:

Concentrated over Past. pstbc. strain No 2^I, the PST-phage lead to a confluent lysis in the liquid area in 39 strains of Past. pstbc. types I -- V, at a dilution of 10^{-3} (highest dilution) or in 3 strains at a dilution of 10^{-2}, or 10^{-1}, Following the concentration over Past. pestis strain A 1122, a confluent lysis was observed in 7 strains (1 strain type I, 4 strains type II, and 2 strains type IV) at a PST-phage suspension up to 10^{-3}, and in 31 strains of types I and V at a concentrated or diluted 1:10 phage suspension. 4 Pst. pstbc. strains of type II with no peculiarities from the cultural or biochemical points of view either showed no reaction to the PST-phage (strain No 784 and 255), or else only the concentrated phage suspension effected numerous or individual plaques in the area of the phage liquid (strain No 792 and 798).

Both PST-phage concentrations, diluted up to 10^{-3}, lead to a confluent lysis in the 3 pestis strains.

Following the transfer of the bacteriophage strain Y to Past. pestis strain A 1122 or Past. pstbc. strain No 2^I and the testing of the two phage suspensions on Past. pstbc., the results shown in the chart below were received.

CHART. Reaction of Past. pstbc. with bacteriophage Y concentrated over Past. pestis and Past. pstbc.

Past. pstbc.		Bacteriophage Y concentrated over	
Serological type	Number of strains	Past. pestis strain A 1122*	Past. pstbc. strain No 2^{I}
		Number of strains showing reaction	
I	13	4	9
	1 (74R)	1	1
II	10	1 (strain No 16)	6
III	5	4	5
IV	4	1 (strain Saisava)	2
V	9	0	5
Total	42	11	28

*At a dilution of 10^{-3}, a confluent lysis in Past. pestis strain A 1122, EV 76, and TWJ.

As the chart shows, the suspensions of bacteriophage Y 11 (types I - IV) or 29 Past. pstbc. strains (types I - V), concentrated over Past. pestis strain A 1122 or Past. pstbc. strain No 2^{I}, showed a reaction. The degree of lytic action of these and other phage suspensions over Past. pstbc. strains No. 1779^{II}, $Ikegaki^{IV}$, and 25^{V}, of the sensitivity of the individual strains of Past. pstbc. types I - V to the various concentrations of the Y-phage was not uniform, so that it was impossible to determine regularities.

Bacteriphage Y, concentrated over Past. pestis strain A 1122, lead to a confluent lysis in only two strains of Past. pstbc. type III (No 2200 and H 21) at a dilution of 10^{-3}, while two other strains of type III, or five of 14 strains of type I, and 1 strain each of types II and IV, showed sporadic plaques only with the concentrated or 1:10 diluted phage suspension. The concentration of bacteriophage Y, gained over Past. pstbc. strain No 2^{I} and diluted up to 10^{-3}, released a confluent lysis in all type III Past. pstbc. strains. This phage concentration produced no reaction in 4 Past. pstbc. strains type I (No 1323, 842, H 19, 1147), 4 of type II (No 255, 256, 257, 798), and 2 strains of type IV (No 32 and Saisava). Consequently, questions II and III may be answered as follows:

The differences in the lytic action of the two PST-pgage concentrations gained over Past. pstbc. strain No 2^{I} or Past. pestis strain A 1122 on Past. pstbc. strains type I - V are primarily quantitative.

With the exception of 2 strains (No 784II and 435II), all Past. pstbc. strains with two different concentrations of the PST-phage strain showed a reaction; however, the titer of the PST-phage suspension over Past. pestis was lower.

The differences in the lytic action of the two Y-phage concentrations on Past. pstbc. strains types I - V are not only quantitative, but also qualitative. A comparison of the action of the two phage concentrations shows that following the concentration of the Y-phage over Past. pstbc. strain No 2I, there was a considerably greater number of Past. pstbc. strains types I - V than with the concentration gained over Past. pestis strain A 1122. The application of the Y-phage to Past. pstbc. strain No 2I, a heterologous strain, lead, as it was to be expected, to an increase of its lytic properties, unlike it was the case with Past. pstbc. strains types I - V.

Tests to Question IV.

We do not intend to go into the individual experiments, since they showed no definite proof of an adaptation of the PST-phage, concentrated separately over Past. pstbc. strain No 16II or 32IV, to S. typhimurium (No 9) and S. schottmuelleri (No 3), or S. enteritidis (No 64) and S. gallinarum pullorum (No 74). In a number of experiments with a filtrate of 20 S. typhimurium cultures on proteose agar plates, not injected with the PST-phage, the same lytic action was observed in 4 of the 21 Salmonella strains tested as in the third filtrate passage of S. typhimurium cultures which had been impregnated with PST-phages, no virulent bacteriophages were found in any of the numerous filtrate passages against Salmonella strains. These observations, however, cannot be generalized as long as we have no further results in other Past. pstbc. bacteriophage strains or Salmonella strains.

Tests on Question V.

A transfer of the Salmonella O1 phage and the Salmonella typhimurium phage No 1 to Past. pstbc. strain No 2I, 16II, 43III, 2200III, IkegakiIV, 32IV, 25V, 74R (rough strain of Past. pstbc. type I; Thal 1954), or to Past. pestis strain A 1122, TWJ, and EV 76, failed in ten and more passages on liquid and solid culture-medium, even with the aid of UV-rays or culture coverings.

Consequently, the initial question regarding the two Salmonella phages used cannot be answered in the affirmative; however, until we have further observations of other Salmonella phages with other experimental techniques, it is impossible to draw definite conclusions.

Summary.

This is a report of studies done with a Past. pstbc. bacteriophage (PST-phage) and a Past. pestis bacteriophage (bacteriophage Y), based on five main points. Under the conditions individually described, the

42 strains of Past. pstbc. type I - V tested reacted to PST-phage, and within the Pasteurella genre 3 pestis strains showed a reaction, while the Past. multocida and Past. tularensis strains showed none. In countries with no occurrence of pestilence, the PST-phage can be a valuable aid for type diagnosis. The concentration of the PST-phage over Past. pestis resulted in a decrease of the lytic action in Past. pstbc. strains without diminishing the number of Past. pstbc. type I - V strains gained. The concentration of bacteriophage Y over Past. pstbc. strains was, on the other hand, a result of the increase in the lytic action and the number of Past. pstbc. Type I-V strains. Definite proof of an adaptation of the PST-phage to Salmonella strains and the Salmonella phage to Past. pstbc. and Past. pestis strains could not be found.

Bibliography.

Adam, M. H.: Bacteriophages. New York: Interscience Publisher 1959.

Advier, M.: Etude d'un bacteriophage antipesteux. (A study of anti-pestis bacteriophage). Bull. Soc. Path. exot. 26, 94-99, (1933).

Bezsonova, A.; P. F. Molodtsova, O. N. Moscolova, B. E. Ye? Oso-linker, A. N. Kraynova, B. A. Pronin, R. I. Timofeyeva, M. S. Shposhnikov, A. G. Sokolinskaya, N. I. Kashkina, A. M. Petrunina, K. A. Goriunova, and A. S. Temiyakova: O diferentsiatsii B. pestis i B. pseudotuberculosis rodentium Pfeiffer a pri pomoshcli chumnovo bacteriofaga. (About the differentiation of B. pestis and B. pseudotuberculosis rodentium pfeiffer with the help of plague-infected bacteriophage) Rev. Microbiol. Saratov 17, 228-231 (1938).

Brandis, H.: Die Anwendung von Phagen in der bakteriologischen Diagnostik mit bes. Berucksichtigung der Typisierung von Typhus- und Paratyphus B-Bakterien sowie Staphylokikken (The application of bacteriophages in bacteriological diagnosis with particular consideration to the differentiation of typhoid and para-typhoid B-bacteria, as well as staphylococci). Ergebn. Mikrobiol. 33, 96 - 159, (1957).

Brandis, H.: Personciche Mitteilungen (Personal reports) 1961.

Flu, P. C.: Sur la nature du bacteriophage (On the nature of bacteriophage), C. R. Soc. Biol., Paris, 96, 1148-1149, (1927).

Flu P., and H. Flu: At n. Leeuwenhoek 11, 195 (1946; quot. according to Pollitzer, R. in Plaque: World Health Org. Series No. 22, p. 171).

Girard, G.: Sur quelques nouyeaux caracteres differenciant les bacilles de la peste et de la pseudotuberculose des pasteurella (Some new characteristics differentiating the pestis bacillus from that of Past. pseudotuberculosis). Ann. Inst. Pasteur 68, 476 - 478, (1942).

Girard, G.: briefliche Mitteilung (letter report) 1960.

Gunnison, J. B., and A. S. Lazarus: Alteration of Past. pestis bacteriophage following successive transfer on Past. pseudotuberculosis and on Shigella, Proc. Soc. exp. Biol., New York, 69, 294-296 (1948).

Gunnison, J. B., A. Larson, and A. S. Lazarus: Rapid differentiation between Past. pestis and Past. pseudotuberculosis by action of

bacteriophage. J. Infect. Dis. 88, 254-255 (1951), with further bibliography.

Gunnison, J. F., M. C. Shevki, V. K. Zion, and M. J. Abbot: Lysis of Past. pseudotuberculosis by bacteriophage. J. infect. Dis. 88, 187-193 (1951).

Kotliarova, R. I.: Pseudo tuberculosis bacteriophage and its characteristics (in Tussian). Trudi nauchno-issledovatelskogo protivochumnogo instituta kavkaza i Zavkakaza (Reports of the Anti-Pestis Institute of the Caucasus and Transcausasia) 1956, pp. 234-241.

Lazarus, A. S. and J. B. Gunnison: The action of Past. pestis bacteriophage on strains of Pasteurella, Salmonella and Shigella. J. Bact. 53, 705-714 (1947).

Plankina, Z. A., and N. S. Ogneva: The Isolation of Pseudotuberculosis Germs in Marmots (In Russian). J. Microbiol. 32, Moscow, 124-127, (1961).

Sugino, T.: On the bacteriophage against the plaque bacillus. Kitasato Arch. exp. Med. 9, 72-81. (1932).

Thal, E.: Untersuchungen uber Past. pseudotuberculosis (Past. Pseudotuberculosis Research). Lund 1954.

- END -